(Invitrogen, Carlsbad, CA) was also prepared for cloning. The digests were purified with WIZARD DNA CLEAN-UP KITS (Promega) according to the manufacturer's recommendations. *iss* was ligated into pVAX1 in 1:1 and 3:1 ratios of molar ends (Sambrook et al. Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York (1989)) using T4 DNA ligase (GIBCO BRL, Gaithersburg, MD). Ligations were transformed into chemically competent bacterial cells (XL-1 Blue, Stratagene, La Jolla, CA). Transformants were selected on kanamycin (50 µg/ml kanamycin, Amresco, Solon, OH). Kanamycin-resistant colonies were transferred to LB broth containing 50 µg/ml kanamycin. After overnight growth, some culture was reserved, and some was subjected to DNA isolation using WIZARD MINIPREPS (Promega). DNA was restricted with Bam*HI* and Xba*I* (New England Biolabs, Beverly, MA). Desired clones were presumptively identified by the size of DNA fragments generated following plasmid restriction. The identity of the insert was confirmed by sequence analysis. The insert region was sequenced using IRD800 labeled T7 promoter/primer (TAATACGACTCACTATAGGG, SEQ ID NO:25) and BGH reverse primer (TAGAAGGCACAGTCGAGG, SEQ ID NO:26) (LI-COR, Lincoln, NE) with the SEQUITHERM EXCEL II (Epicentre, Madison, WI) cycle sequencing kit on a LI-COR 4000LS automated DNA sequencer. The *iss* vaccine construct can be seen in Figure 6.

In the Claims

Please cancel claims 1-29 and 34, without prejudice. Please amend claims 30-33, 35-37 and 43-45. The new and amended claims are provided below in clean form. Pursuant to 37 C.F.R. §1.121, the amended claims are also shown in Appendix A with notations to indicate changes made (for convenience, all pending claims are provided in Appendix A).

30. (Amended) An isolated nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

31. (Amended) The isolated nucleic acid molecule of claim 30 further comprising nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21, wherein the 33 nucleotides of the nucleotide sequence SEQ ID NO:21 are located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

Sub B1

32. (Amended) The isolated nucleic acid molecule of claim 30 wherein nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22 is operably linked to a promoter functional in a host cell so as to form an expression vector.

B19

33. (Amended) An expression vector comprising an isolated nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22, operably linked to at least one regulatory sequence or control sequence.

35. (Amended) A method of using a nucleic acid molecule encoding an *E. coli* Iss polypeptide, the method comprising:

providing a host cell stably transformed with an expression vector comprising a nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22, operably linked to a least one regulatory sequence or control sequence ecognized by the host cell; and

B20

expressing the nucleic acid molecule to yield an *E. coli* Iss polypeptide.

36. (Amended) The method of claim 35 wherein the nucleic acid molecule further comprises nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21 located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

Sub B21

Sub B4 B20

37. (Amended) An immunogenic composition comprising:

a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an avian *E. coli* Iss polypeptide or an immunogenic fragment or immunogenic subunit thereof; and

a pharmaceutically acceptable carrier.

Sub B4 B21

43. (Amended) The immunogenic composition of claim 38 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22 or an immunogenic subunit or immunogenic fragment thereof.

44. (Amended) The immunogenic composition of claim 38 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

45. (Amended) The immunogenic composition of claim 43 wherein the nucleic acid molecule further comprising nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21 located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

Please add the following new claim:

B22 Sub B21

67. (New) The immunogenic composition of claim 43 wherein the immunogenic composition generates an antibody response when administered to a subject.